

*The Antiseptic Action of Substances of the Chloramine Group.**

By H. D. DAKIN, J. B. COHEN, F.R.S., M. DAUFRESNE, and J. KENYON.

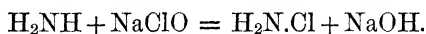
(Report to the Medical Research Committee.)

(Received February 5, 1916.)

In the course of experiments relating to the employment of antiseptics in the treatment of infected wounds, one of the present writers introduced a modification of the ordinary sodium hypochlorite solution which was found capable of giving useful results when properly employed.† The advantages of this sodium hypochlorite preparation for the treatment of infected wounds compared with some commonly used antiseptics may be referred to the following properties—though powerfully germicidal it does not coagulate blood serum or other protein substances, while at the same time it dissolves necrotic tissue; it is freely soluble and can penetrate to a certain extent, and when properly prepared it is practically non-irritating at 0·5 per cent. concentration.

Out of a very large number of antiseptics that were systematically examined, the hypochlorites seemed to be among the most generally useful. It was, therefore, of interest to study the mode of action of hypochlorites and to endeavour to find related substances which might prove to be of greater practical value.

A number of years ago Raschig showed that when a hypochlorite solution is added to ammonia, the simplest chloramine, NH_2Cl , is formed



This reaction between ammonia and hypochlorite is typical of many similar changes between hypochlorous acid or hypochlorites on the one hand, and more complex amino-compounds on the other. Our knowledge of these changes is chiefly due to the extensive researches of Chattaway. A great variety of organic substances containing (NH) groups react with hypochlorites to give compounds of the chloramine group, *i.e.*, substances containing the :NCl radical.

It appears probable that the germicidal action of hypochlorites is due to chemical reactions of a similar type. It is reasonable to assume that the killing of micro-organisms by antiseptics is due to chemical changes brought

* The work reported in this communication was done partly at the University of Leeds, with the support of the Medical Research Committee, and partly at Hospital 21, Compiègne, France, in laboratories supported by the Rockefeller Institute.

† 'Brit. Med. Journ.,' August 28, October 23, November 27, and December 4, 1915.

about in some of the compounds of the living cell, either by direct action of the antiseptic or by the action of products formed from the antiseptic by combination with substances present in the medium in which the organisms are suspended. Among the chemical substances present in living cells capable of reacting with hypochlorites, the proteins appear likely to play a dominant *role*. Under conditions of practical use antiseptics commonly act upon organisms suspended in a medium more or less rich in proteins. Hence hypochlorites might be expected to react on both intra- and extra-cellular proteins.

The action of hypochlorites upon proteins consists, at least in part, in the replacement of the hydrogen of some of the (NH) groups by chlorine, thus forming substances of the chloramine group.* The reaction is somewhat complicated by other changes which will be referred to in more detail later. Now the ability of hypochlorites to attack proteins with the formation of substances containing halogen directly linked to nitrogen appears to be related to their bactericidal action. One piece of indirect evidence may be quoted at once. Free chlorine, bromine, and iodine have not widely differing germicidal power, but when the halogen is converted into hypochlorite or hypobromite, a very marked difference appears. The germicidal action of the hypochlorites, when tested against staphylococci suspended in water, is of a similar order to that of free chlorine, that of hypobromite is only about 1 per cent. of that of free bromine, while a solution of iodine in weak alkali, which may contain unstable hypo-iodite, has a negligible germicidal activity. This feeble germicidal action of hypobromite and hypo-iodite may well be related to their known sluggishness in reacting with proteins and amino-acids compared with the comparative activity of the hypochlorites.† The probability that the formation from proteins of substances containing halogen linked to nitrogen was an intermediate agent in the germicidal action of hypochlorites made it desirable to make a systematic investigation of the germicidal properties of a large number of substances of varied type containing the same (NCl) group. Most of these substances, as will be seen

* Hopkins and Pinkus ('Ber.,' vol. 31, p. 1311, 1898) showed that proteins which had been treated with a large excess of chlorine contained part of the halogen in a loosely combined form capable of liberating iodine from potassium iodide. The view that these substances contained chlorine attached to nitrogen was first put forward by Cross, Bevan, and Briggs ('Journ. Soc. Chem. Ind.,' vol. 27, p. 260, 1908). In a previous communication by one of us (H. D. D.) this paper was unfortunately overlooked.

† Compare Struhetz ('Monatsh.,' vol. 27, p. 601, 1906). Langheld ('Ber.,' vol. 42, p. 2360, 1909) states that gelatine is not attacked by cold sodium hypochlorite. This erroneous conclusion is apparently based on the observation that on mixing the two solutions, unchanged hypochlorite persists, but as a matter of fact the bulk of the hypochlorite readily reacts with the gelatine.

from the Table of Results (p. 243), possess powerful germicidal properties. It is worthy of note that, as shown in the case of the derivatives of acylanilides, marked germicidal properties only appear on the introduction of chlorine attached to nitrogen. The parent substances from which these chloramines are prepared, whether unsubstituted or containing chlorine attached to carbon, show no such action.

The bacteriological tests were made by determining the approximate concentration necessary to sterilise completely one or two drops of a 24-hour-old culture of a vigorous strain of *Staphylococcus aureus* in a total volume of 5 c.c. In a number of cases other suitable organisms, especially *pyocyaneus*, were used as well. Two sets of tests were made, one in which the organisms were suspended in water, the other in which the fluid contained 50 per cent. of horse serum, as it is well known that the presence of serum or other proteins markedly inhibits the action of all known antiseptics. The practical details are noted later.

The substances tested include the following groups:—

- | | |
|--------|---|
| Series | I.—Chloramine compounds in which the NCl group is separated from a benzene nucleus by the SO ₂ Na group. |
| „ | II.—Naphthalene derivatives similar to the above. |
| „ | III.—Other dicyclic derivatives of similar type. |
| „ | IV.—Chloramines in which the NCl group is directly attached to a benzene nucleus. |
| „ | V.—Bromamines. |
| „ | VI.—Products of the action of hypochlorite on proteins, etc. |
| „ | VII.—Certain other compounds for comparison. |

A superficial consideration of the results contained in the above Table at once permits certain simple deductions:—

(1) Almost all of the substances examined containing the (NCl) group possess very strong germicidal action.

(2) The presence in the molecule of more than one (NCl) group does not confer any marked increase in germicidal power; compare substances 20, 24, 25, and 26.

(3) The germicidal action of many of these chloramine compounds is molecule for molecule greater than that of sodium hypochlorite.* Thus *p*-toluene sodium sulphochloramide with a molecular weight for the crystallised salt of 261.5 is as active as sodium hypochlorite with a molecular weight of 74.

* Rideal ('Journ. Roy. San. Inst.,' vol. 31, p. 33, 1910) had shown previously, that addition of ammonia to hypochlorite with formation of NH₂Cl can lead to an increase over the original germicidal action of the original hypochlorite.

(4) Substitution in the nucleus of aromatic chloramines by Cl, Br, I, CH₃, C₂H₅, or NO₂ groups does not lead to any very great increase in germicidal activity. More commonly there is a moderate diminution.

(5) The chloramine derivatives of naphthalene and other dicyclic compounds of the sulphochloramide type closely resemble the simpler aromatic chloramines in germicidal action.

(6) The few bromamines examined show a slightly lower germicidal action than the corresponding chloramines. But the sodium sulphobromamides are much more active than sodium hypobromite. It is significant that they react much more readily with amino-acids and proteins than does sodium hypobromite.

(7) Derivatives of proteins prepared by the action of sodium hypochlorite and containing (NCl) groups are strongly germicidal. Blood serum inhibits their germicidal action to much the same extent as it does with sodium hypochlorite or the aromatic chloramines.

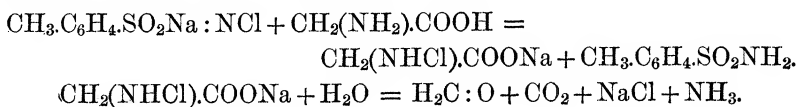
Mode of Action of Chloramines and Related Substances.

Although it is obvious that nothing more than an incomplete explanation is at present feasible, it is possible to discuss some of the factors regulating the germicidal action of the chloramines.

Chloramines and bromamines are usually more actively germicidal—or at least will kill organisms at a lower molecular concentration—than the corresponding hypochlorite or hypobromite. Hence they cannot be regarded as the biochemical equivalents of the latter substances. It appears as if their germicidal qualities were due to the properties of the complex chloramine or bromamine molecule as a whole, apart from the action of the active halogen in them.

But, on the other hand, we find that chloramines—at any rate, those of the aromatic sodium sulphochloramide type—react with amino-acids, peptones, proteins, and many other substances in very much the same fashion as sodium hypochlorite.

Thus, for example, on adding *p*-toluene sodium sulphochloramide to an aqueous solution of an amino-acid, toluene sulphonamide is precipitated, and the amino-acid is converted into an unstable chloramino-acid, which subsequently decomposes to give an aldehyde and carbon dioxide. In the case of glycine, the reaction may be represented as follows :—

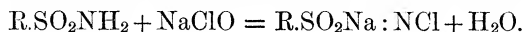


This change is very similar to the reaction between amino-acids and sodium hypochlorite investigated by Langheld.*

Peptones and proteins react with *p*-toluene sodium sulphochloramide rather more slowly than the amino-acids, judged by the rate of separation of the sparingly soluble toluene sulphonamide. Ammonium salts react rapidly with the sulphochloramide, urea more slowly, while hippuric acid, and probably other acyl-amino-acids, do not react appreciably. These changes in each case resemble the action of hypochlorite. It should be noted, too, that Langheld found that the simplest chloramine, NH_2Cl , reacts with amino-acids like hypochlorite. Judging by these reactions of chloramines, such as toluene sodium sulphochloramide, upon amino-acids, peptones, proteins, ammonium salts, urea, and related substances, it is clear that chloramines can act as chlorinating agents upon important constituents of living cells.

It appears that the aromatic sulphochloramides act as chlorinating agents only when there is opportunity for the chlorine to leave the chloramine and attach itself to nitrogen in a second compound in which the nitrogen atom is united to less acid groups than those in the original substance.

We have found that the aromatic sodium sulphochloramides are readily formed by the addition of sodium hypochlorite to aromatic sulphonamides :



Since the reactions of the resulting products so closely resemble those of hypochlorites, it might be thought that the above reaction was a reversible one. That this is not the case follows from the following observations:—
(a) Aqueous solutions of many of the sulphonamides are remarkably stable. Toluene sodium sulphochloramide solutions retain their strength unchanged for months, while if neutral hypochlorite were present, decomposition would be rapid. (b) Chloramine solutions do not give the reaction with aniline, indigo, etc., characteristic of hypochlorites.

From the foregoing observations the following tentative conclusions may be drawn:—The fact that many of the constituents of living organisms, including proteins, peptones, amino-acids, urea, and ammonium salts, contain nitrogen in a form capable of attracting the chlorine from chloramines of different kinds, is probably connected with the germicidal action of the latter group of substances. On the other hand, while the chlorinating action of chloramines resembles that of hypochlorites, the germicidal action of the chloramines is often greater than that of an equivalent amount of hypochlorite. This superior germicidal action can be ascribed either to some

* 'Ber.,' vol. 42, p. 2360 (1909).

obscure special action of the chloramine molecule as a whole or possibly to selective chlorination of particular cell constituents.

Practical Use of Chloramines as Antiseptics.—In judging of the suitability of any particular substance for practical use as an antiseptic many factors need consideration. High germicidal activity, when tested against micro-organisms suspended in water is, of course, desirable, but the degree to which this activity is reduced by blood serum, pus, and similar substances is of greater importance, since many antiseptics which act efficiently in watery solutions are practically inert when used on an infected wound. The question of protein coagulation by antiseptics appears to be important, and the fact that hypochlorites and chloramines, unlike many common antiseptics, are not protein coagulants under conditions of practical use is greatly in their favour. Solubility is another important factor. Many of the chloramines, especially those prepared from acylanilides (Section IV in Table), have high germicidal action, but are very sparingly soluble in water. They may be dissolved in vaseline or lanoline, and many of these mixtures were given practical trials. They proved to be of little value, for organisms grew readily under the fatty film. They were deficient in penetrating power, and their use was discontinued. We were therefore led to pay special attention to the chloramines capable of forming soluble sodium salts, and some of these substances appear to be of genuine value.

On the whole, *p*-toluene sodium sulphochloramide seems to be as good as any, and it has already been used successfully for a variety of purposes. The practical applications of the compound have been dealt with elsewhere.* The substance is easily and cheaply made, especially by a method described in the practical part of this paper. It is relatively non-irritating to wounds and can be used in situations where the ordinary antiseptics would prove too irritating or deficient in germicidal action. It may be noted that this compound is readily made from *p*-toluene sulphonic chloride, a cheap by-product in the manufacture of saccharin. Its manufacture has been undertaken by several British firms, and is sold under the name Chloramine-T.

Reference may be made at this point to a curious compound of hexamethylenetetramine and calcium hypochlorite. It is readily precipitated in the form of fine crystals on adding strong bleaching powder solution to hexamethylenetetramine. The compound has strong antiseptic properties, but is of no practical use, since on keeping in the dry state for a few weeks, it decomposes completely. Substances of this type do not appear to have been previously described. Reference may be made to Delepine's experiments on the action of sodium hypochlorite on hexamethylenetetramine. In

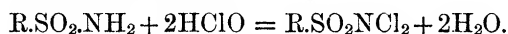
* 'Brit. Med. Journ.,' January 28, 1916.

neutral solution N-dichloropentamethylenetetramine is obtained, while in acetic acid solution trichlorohexahydrotriazine is formed.*

Practical.

Preparation of Substances.—The action of sodium hypochlorite on the sulphonamides was first examined by Raper, Thompson and Cohen.† Later, Chattaway, by a similar reaction, prepared a series of sulphochloramides, details of which can be found without difficulty in the 'Trans. Chem. Soc.' Reference will be made here simply to substances which had not been hitherto obtained by others, or for the preparation of which improved methods have been adopted.

A number of new sulphochloramides were prepared from the corresponding hydrocarbons by successive formation of the sulphonic acid, sulphonic chloride, sulphonamide, and sulphodichloramine. The latter substances were converted into the sodium sulphochloramides by the action of sodium hydroxide according to Chattaway's method.‡



The sodium sulphochloramides of the following hydrocarbons had not been previously obtained: *o*-, *m*- and *p*-xylene, ethylbenzene, chlorobenzene,§ dichlorobenzene, bromobenzene, iodobenzene, mesitylene, pseudocumene, dibenzyl. They are all soluble crystalline salts, sparingly soluble in caustic soda solution, and give the general reactions for chloramines. The sodium salts of the sulphochloramides of diphenyl and diphenylmethane, and 2.6 naphthalene sodium sulphochloramide were obtained in solution by decomposing the corresponding dichloramines with hot sodium hydroxide and then neutralising the solution.

We have found that a number of sodium sulphochloramides, including those of benzene and toluene, may be conveniently and economically made by a process which avoids the intermediate preparation of the sulphodichloramine. This method simply consists in dissolving the powdered sulphonamide (1 mol.) in a cold solution of sodium hypochlorite (1.2 mols.), warming and filtering if necessary, and then adding one and a half volumes of saturated brine. The sodium salt of the sulphochloramide crystallises out

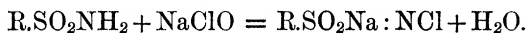
* 'Bull. Soc. Chim.' (iv), vol. 9, p. 1025 (1911).

† 'Trans. Chem. Soc.', vol. 85, p. 371 (1904).

‡ 'Trans. Chem. Soc.', vol. 87, p. 145 (1905).

§ Since this work was completed, Chattaway has described the three mono-halogen benzene sodium sulphochloramides. 'Trans. Chem. Soc.' vol. 107, p. 1814 (1915).

of solution in about 90 per cent. yield, and is washed with brine and dried in the air.



The products of the action of sodium hypochlorite on proteins referred to in the Table can hardly be regarded as chemical individuals. They were prepared by adding an excess of hypochlorite, with or without addition of sodium bicarbonate, to a solution of the protein. After a short time the chlorinated product was precipitated either by acidification or by salting out with magnesium or sodium sulphate.

When an excess of sodium hypochlorite is added to blood serum, a certain proportion of the hypochlorite rapidly disappears owing probably to substitution of hydrogen attached to carbon in the various cyclic rings, *e.g.*, tyrosine, tryptophane, histidine. Another portion of the hypochlorite replaces hydrogen attached to nitrogen, and the resulting protein-like substances containing NCl groups when precipitated and washed free from adhering hypochlorite liberate iodine promptly from hydriodic acid. In addition a considerable amount of hydrolysis takes place in the protein-hypochlorite mixture and a certain amount of nitrogen gas is evolved. The formation of aldehydes by the decomposition of the chloramino-acids is also easily noticeable. The reaction between proteins and hypochlorites is a complicated one and will be the subject of further investigation.

The compound of calcium hypochlorite and hexamethylenetetramine previously referred to was prepared as follows:—A clear filtered solution obtained from 50 grm. of good bleaching powder and 200 c.c. of water is added to a solution of 20 grm. of hexamethylenetetramine in 40 c.c. of water. The mixture is at once cooled, when needle-shaped crystals are deposited and fill the liquid. The crystals are filtered off, washed with a little cold water and dried rapidly in *vacuo*. The yield is 14 grm. The calcium in this compound can be removed by oxalic acid or carbon dioxide, giving a faintly green solution with marked bleaching properties. The substance decomposes rapidly on keeping for two or three weeks.

Analyses of freshly prepared specimens showed that almost all the chlorine was in the hypochlorite form and that the ratio of chlorine to nitrogen was exactly 2:4. The proportion of calcium in the undehydrated substance (14.7 per cent.) was always too high for the simple formula



The ability of hexamethylenetetramine to form complex salts is well known, and this may account for the excess of calcium.

Determination of Germicidal Action.—The antiseptic action of the substances referred to in the Table was characterised by determining the minimal concentration of a solution capable of killing in two hours at the temperature of the laboratory the organisms under investigation, suspended either in water or in serum. The technique was as follows:

A series of tubes each containing 5 c.c. of a solution of the substance at a progressively decreasing concentration is first of all prepared, and to each tube one or two drops of a 24-hour-old culture of the organism in peptone bouillon is added. A blank experiment with 5 c.c. of distilled water with a drop of the culture is also made. The mixtures of substance and microbes are carefully shaken every half hour and remain at a temperature of 18–20° for two hours. A loop full of the contents of each tube is then taken and added to a series of tubes, each containing 3 c.c. of bouillon, which are then incubated for 24 hours at 37°. When no growth appears in these inoculated tubes, the concentration of the antiseptic in the first series of tubes is judged sufficient to kill the organisms. Incomplete sterilisation is indicated by growth, and the necessary concentration for sterilisation can be determined more closely by subsequent repetitions at less widely varying strengths of solution.

The tests carried out in the presence of blood serum were performed in the same way, only the liquid contained in the first series of tubes contained 50 per cent. of horse serum previously heated to 55–56°. One drop of culture was added for every 2 c.c. of the serum mixture.

Action of Chloramines on Amino-acids.—The chloramine employed in these experiments was usually *p*-toluene sulphochloramide. When an aqueous solution of this substance is added to a solution of an amino-acid, *p*-toluene sulphonamide is rapidly precipitated, and a pungent odour, probably due to a chloramino-acid, is noted. On standing or on warming the solution, carbon dioxide is rapidly evolved through the decomposition of the chloramino-acid, and an aldehyde is formed. In the following experiments equimolecular quantities of the amino-acid and toluene sulphonamide in water were mixed and then distilled.

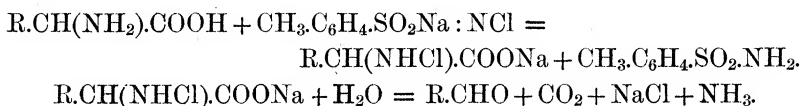
Glycine gave formaldehyde, which was characterised by its nitrophenyl-hydrazone, M.P. 181°, by conversion into hexamethylenetetramine on evaporation with ammonia, and by various colour reactions.

Glycine anhydride behaved similarly to the above.

Alanine gave acetaldehyde, which was identified by conversion into the nitrophenylhydrazone, which, after crystallisation from alcohol, melted at the correct temperature, 130°. The Rimini reaction with sodium nitroprusside and piperidine was strongly positive.

Leucine gave oily isovaleric aldehyde which was identified by conversion into the nitro-phenylhydrazone.

Amino-phenylacetic acid and *methylamino-phenylacetic acid* both gave large quantities of benzaldehyde, which for purposes of identification was converted into the phenylhydrazone, crystallising from alcohol in needles, M.P. 159°, and into the nitro-phenylhydrazone, M.P. 192°. In each case the aldehyde obtained contained one less carbon atom than the amino-acid taken, and it is clear that the reaction proceeds according to the following scheme :—



The similarity between this reaction and the oxidation of amino-acids with hypochlorite has already been noted.

Tryptophane.—When *p*-toluene sulphochloramide is added to tryptophane a blue-violet substance soluble in amyl alcohol, and closely resembling the coloured product of the Hopkins glyoxylic acid reaction is obtained.

Blood Serum.—When chloramine dissolved in water is left in contact with blood serum at the ordinary temperature for 2–3 weeks and the product distilled, the distillate gives the colour reactions for both formaldehyde and acetaldehyde, and a crystalline phenylhydrazone, melting indefinitely at 135–150°, which is probably a mixture of the phenylhydrazones of the two aldehydes (130° and 178°).

Stability of p-Toluene Sodium Sulphochloramide.—Reference has already been made to the remarkable stability of toluene sodium sulphochloramide in solution as evidence of the non-dissociation of sodium sulphochloramides into sulphonamides and sodium hypochlorite. The following Table contains the results of some of these observations, which were made with *p*-toluene sodium sulphochloramide. This substance was chosen since, on account of its practical applications, the information was of special value. The concentrations of the solutions were determined by titration with decinormal sodium thiosulphate after addition of potassium iodide and acetic acid. It will be seen that the rate of decomposition of the solutions in the dark is inappreciable after 132 days, while solutions exposed to daylight showed a slight but trivial loss of strength. Two other points which may be of practical value may be mentioned. *p*-Toluene sodium sulphochloramide should not be left in long contact with steel instruments, since, as would be expected, the metal is attacked. In order to see if clean surgical gauze would cause decomposition of the sulphochloramide, a solution containing

1·72 grm. in 50 c.c. was added to 1 square foot of gauze. The decomposition was only 0·05 grm. in 123 days, and the gauze itself was not damaged in any way.

Days.....	0.	1.	2.	8.	16.	23.	51.	123.
In light	0·832	0·818	0·812	0·795	0·792	0·782	0·775	0·756
„	1·653	1·639	1·639	1·608	1·596	1·579	1·543	1·540
„	3·282	3·282	3·277	3·225	3·193	3·192	3·086	3·080
In dark	0·826	0·815	0·815	0·815	0·815	0·815	0·815	0·815
„	1·648	1·648	1·648	1·648	1·642	1·642	1·642	1·642
„ at 37° C.	1·648	1·648	1·648	1·648	1·648	1·648	1·648	1·648
„	3·311	3·311	3·294	3·300	3·294	3·294	3·311	3·282

The figures in the above Table represent grammes per 100 c.c. of solution.

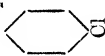
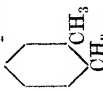
TABLE OF RESULTS.

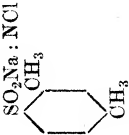
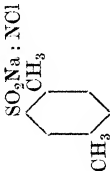
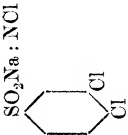
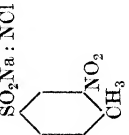
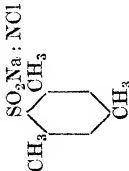
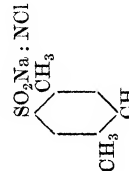
No.	Name.	Formula.	Staphylococci tests.		Pyocyanus tests.	
			In water.	In serum.	In water.	In serum.
SERIES I.						
1	Benzene sodium sulphochloramide*	$\text{SO}_2\text{Na}:\text{NCl}$ 	1 : 250,000— 1 : 500,000 +	1 : 1000— 1 : 2500 +	1 : 50,000— 1 : 1,000,000 +	1 : 500— 1 : 1000 +
2	<i>o</i> -Toluene sodium sulphochloramide	$\text{SO}_2\text{Na}:\text{NCl}$ 	1 : 500,000— 1 : 1,000,000 +	1 : 500— 1 : 1000 +		
3	<i>p</i> -Toluene sodium sulphochloramide†	$\text{SO}_2\text{Na}:\text{NCl}$ 	1 : 500,000— 1 : 1,000,000 +	1 : 1500— 1 : 2500 +	1 : 200,000— 1 : 500,000 +	1 : 1000— 1 : 2000 +
4	<i>p</i> -Ethylbenzene sodium sulphochloramide	$\text{SO}_2\text{Na}:\text{NCl}$ 	1 : 100,000— 1 : 250,000 +	1 : 1000— 1 : 2500 +	1 : 100,000— 1 : 250,000 +	1 : 500— 1 : 1000 +

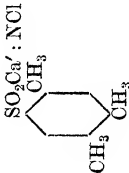
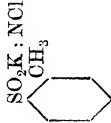
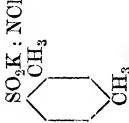
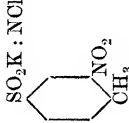
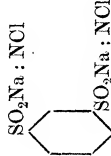
* Tested against streptococci, this substance gives the following results: in water, 1 : 500,000—, 1 : 1,000,000+; in serum, 1 : 1000—, 1 : 5000+.

Against *B. coli* and *B. typhosus*: in water, 1 : 50,000—, 1 : 100,000+; in serum, 1 : 500—, 1 : 1000+.

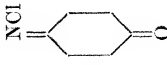
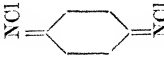
† Streptococci: in water, 1 : 10,000,000—; in serum, 1 : 2500—, 1 : 5000+. With *B. capsulatus*, the results are the same.

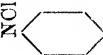
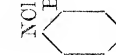
No.	Name.	Formula.	Staphylococci tests.		Pyocyanus tests.	
			In water.	In serum.	In water.	In serum.
SERIES I—continued.						
5	<i>p</i> -Chlorobenzene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000 — 1 : 250,000 +	1 : 1000 — 1 : 2500 +	1 : 50,000 — 1 : 100,000 +	1 : 500 — 1 : 1000 +
6	<i>p</i> -Bromobenzene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000 — 1 : 250,000 +	1 : 500 — 1 : 1000 +	1 : 10,000 — 1 : 50,000 +	1 : 500 — 1 : 1000 +
7	<i>p</i> -Iodobenzene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000 — 1 : 250,000 +	1 : 1000 — 1 : 2500 +	1 : 25,000 — 1 : 50,000 +	1 : 500 — 1 : 1000 +
8	<i>m</i> -Nitrobenzene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 25,000 — 1 : 50,000 +	1 : 500 — 1 : 1500 +	1 : 10,000 — 1 : 25,000 +	1 : 500 — 1 : 1000 +
9	<i>o</i> -Xylene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000 — 1 : 250,000 +	1 : 500 — 1 : 1000 +	1 : 25,000 — 1 : 50,000 +	1 : 500 +

10	<i>m</i> -Xylene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
11	<i>p</i> -Xylene sodium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +	1 : 100,000 – 1 : 250,000 +	1 : 250 – 1 : 500 +
12	<i>o</i> -Dichlorobenzene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 25,000 – 1 : 50,000 +	1 : 500 – 1 : 1000 +
13	<i>o</i> -Nitrotoluene sodium sulphochloramide		1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 100 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
14	Mesitylene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +
15	Pseudocumene sodium sulphochloramide		1 : 100,000 – 1 : 250,000 +	1 : 1000 – 1 : 2500 +	1 : 50,000 – 1 : 100,000 +	1 : 500 – 1 : 1000 +

No.	Name.	Formula.	Staphylococci tests.		Pyocyanus tests.	
			In water.	In serum.	In water.	In serum.
SERIES I—continued.						
16	Pseudocumene calcium sulphochloramide		1 : 50,000 — 1 : 100,000 +	1 : 1000 — 1 : 2500 +	1 : 50,000 — 1 : 100,000 +	1 : 250 — 1 : 500 +
17	<i>o</i> -Toluene potassium sulphochloramide		1 : 10,000 — 1 : 100,000 +	1 : 250 +		
18	<i>m</i> -Xylene potassium sulphochloramide		1 : 10,000 +	1 : 250 +		
19	<i>o</i> -Nitrotoluene potassium sulphochloramide		1 : 50,000 — 1 : 100,000 +	1 : 250 — 1 : 500 +		
20	Benzene sodium disulphochloramide		1 : 100,000 — 1 : 1,000,000 +	1 : 1000 — 1 : 2500 +	1 : 100,000 — 1 : 1,000,000 +	1 : 1000 — 1 : 2500 +

21	Benzyl sodium sulphochloramide	$\text{CH}_2\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000— 1 : 250,000 +	1 : 500— 1 : 1000 +	1 : 50,000— 1 : 100,000 +	1 : 500— 1 : 1000 +
SERIES II.						
22	α -Naphthalene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 250,000— 1 : 500,000 +	1 : 1000— 1 : 2500 +		
23	β -Naphthalene sodium sulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 100,000— 1 : 250,000 +	1 : 500— 1 : 1000 +		
24	1 : 4 Naphthalene sodium disulphochloramide	$\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 10,000— 1 : 50,000 +	1 : 500— 1 : 1000 +	1 : 10,000— 1 : 50,000 +	1 : 500— 1 : 1000 +
25	2 : 6 Naphthalene sodium disulphochloramide	$\text{ClN} : \text{NaO}_2\text{S}$ $\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 250,000— 1 : 500,000 +	1 : 500— 1 : 1000 +	1 : 250,000— 1 : 500,000 +	1 : 500— 1 : 1000 +
26	2 : 7 Naphthalene sodium disulphochloramide	$\text{ClN} : \text{NaO}_2\text{S}$ $\text{SO}_2\text{Na} : \text{NCl}$ 	1 : 250,000— 1 : 500,000 +	1 : 250— 1 : 500 +	1 : 100,000— 1 : 250,000 +	1 : 250— 1 : 500 +
27	Sodium o-sulphochloramidobenzoate	$\text{SO}_2\text{Na} : \text{NCl}$ COOH 	1 : 100,000— 1 : 500,000 +	1 : 250— 1 : 500 +		

No.	Name.	Formula.	Staphylococci tests.		Pyocyanus tests.	
			In water.	In serum.	In water.	In serum.
SERIES III—continued.						
28	<i>p</i> -Diphenyl sodium disulphochloramide*	$\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$ $\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$	1:250,000— 1:500,000+	1:250— 1:500+	1:250,000— 1:500,000+	1:500— 1:1000+
29	Diphenylmethane sodium disulphochloramide*	$\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$ CH_2 $\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$	1:250,000— 1:500,000+	1:500— 1:1000+	1:250,000— 1:500,000+	1:1000— 1:2500+
30	Dibenzyl sodium disulphochloramide*	$\text{CH}_2\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$ $\text{CH}_2\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}:\text{NCl}$	1:50,000— 1:100,000+	1:250— 1:500+	1:50,000— 1:100,000+	1:250— 1:500+
SERIES IV.						
31	Quinone chlorimide		1:50,000— 1:100,000+	1:5000— 1:10,000+		
32	Quinone dichlorimide		1:1000— 1:10,000+			

33	Acetylchloraminobenzene†		1 : 100,000 — 1 : 500,000 +		
34	Acetylchloramino- <i>p</i> -chlorobenzene		1 : 100,000 — 1 : 500,000 +		
35	Formylchloramino-2 : 4-dichlorobenzene		1 : 100,000 — 1 : 500,000 +		
36	Acetylchloramino-2 : 4-dichlorobenzene‡		1 : 200,000 — 1 : 500,000 +	1 : 500 — 1 : 1000 +	1 : 1000 — 1 : 2500 +
37	Acetylchloramino-2 : 4-dibromobenzene		1 : 100,000 — 1 : 500,000 +	1 : 500 — 1 : 1000 +	1 : 500 — 1 : 1000 +

* These substances were prepared for us by the kindness of Mr. Joseph Marshall.

† In comparing the results of this and following substances, it should be noted that the related compounds in which chlorine is not attached to nitrogen, namely, formamide, acetamide, *p*-chloroformamide, *p*-chloroacetamide, and 2 : 4 dichloroacetamide show no marked germicidal properties. None of them kill staphylococci in water at much under 1 : 200 concentration.

‡ Streptococci : in water, 1 : 10,000,000 — ; in serum, 1 : 2500 —, 1 : 5000 +.

No.	Name.	Formula.	Staphylococci tests.		Pyocyanus tests.	
			In water.	In serum.	In water.	In serum.
SERIES IV—continued.						
38	Acetylchloramino-chloroxylene		1 : 100,000 — 1 : 250,000 +	1 : 2000 — 1 : 5000 +		
39	Diacetylchloramino-dichlorobenzene		1 : 100,000 — 1 : 250,000 +	1 : 1000 — 1 : 2500 +	1 : 100,000 — 1 : 250,000 +	1 : 1000 — 1 : 2500 +
SERIES V.						
40	Benzobromamide		1 : 100 — 1 : 1000 +			
41	Benzene sodium sulphobromamide		1 : 50,000 — 1 : 100,000 +	1 : 250 — 1 : 500 +		
42	p-Xylene sodium sulphobromamide		1 : 50,000 — 1 : 100,000 +	1 : 100 — 1 : 250 +	1 : 50,000 — 1 : 100,000 +	1 : 100 — 1 : 250 —

SERIES VI.					
43	Chlorinated blood albumin	—	1 : 250,000 — 1 : 500,000 +	1 : 1000 — 1 : 2500 +	1 : 2500 — 1 : 5000 +
44	Chlorinated egg albumin	—	1 : 100,000 — 1 : 500,000 +	1 : 1000 — 1 : 2500 +	1 : 500,000 — 1 : 1,000,000 +
45	Chlorinated casein	—	1 : 75,000 — 1 : 150,000 +	1 : 1000 — 1 : 2500 +	
46	Chlorinated peptones	—	1 : 50,000 — 1 : 100,000 +	1 : 500 — 1 : 1000 +	
SERIES VII.					
48	Sodium hypochlorite	NaClO	1 : 500,000 — 1 : 1,000,000 +	1 : 1500 — 1 : 2000 +	1 : 2500 — 1 : 5000 +
49	Sodium hypobromite	NaBrO	1 : 2500 — 1 : 5000 +		
50	Iodine	I	1 : 250,000 — 1 : 500,000 +	1 : 1000 — 1 : 2500 +	
51	Iodine dissolved in weak sodium hydrate	—	1 : 100 — 1 : 250 +		
52	Compound of hexamethylenetetramine and calcium hypochlorite	—	1 : 250,000 — 1 : 500,000 +	1 : 1000 — 1 : 2500 +	